Analysis of a mathematical model of syntrophic bacteria in a chemostat

Tewfik Sari, Miled El Hajji, Jérôme Harmand

ANALYSIS OF A MATHEMATICAL MODEL OF SYNTROPHIC BACTERIA IN A CHEMOSTAT

TEWFIK SARI, MILED EL-HAJJI, JÉRÔME HARMAND

Abstract. A mathematical model involving a syntrophic relationship between two populations of bacteria in a continuous culture is proposed. A detailed qualitative analysis is carried out. The local and global stability analysis of the equilibria are performed. We demonstrate, under general assumptions of monotonicity, relevant from an applied point of view, the asymptotic stability of the positive equilibrium point which corresponds to the coexistence of the two bacteria. A syntrophic relationship in the anaerobic digestion process is proposed as a real candidate for this model.

1. Introduction

A synthrophic relationship between two organisms refers to a situation where the species exhibit mutualistism but where, at the opposite of what happens in a purely symbiotic relationship, one of the species can grow without the other. Such a situation can be mathematically formalized as follows. Assume that a first species denoted X_1 grows on a substrate S_1 forming an intermediate product S_2. This intermediate product is required by a second species X_2 to grow. The limiting substrate of the second bacteria being the product of the first bioreaction, the second bacteria cannot grow if the first one is not present.

Such interactions are quite common in nature: it is why a number of models have already been proposed in the literature. Katsuyama *et al.* [9], proposed a model involving two mutualistic species for describing pesticide degradation, while a more general case is considered by Kreikenbohm and Bohl [10]. Since mutualism involves generally species interacting through intermediate products, other studies consider mutualistic relationships in food webs. For instance, Bratbak and Thingstad [2], or more recently, Aota and Nakajima [1] considered the mutualism between phytoplankton and bacteria through the carbon excretion by the phytoplankton. A model studied by Freedman *et al.* [8] was proposed to explain the observed coexistence of such species. However, in the previous studies the models are very specific. In particular, the mathematical analyses of the models are realized for specific growth rates that are explicitely given (in most cases as Monod functions).

To extend the study of mutualism to more general systems, we have recently considered more general assumptions notably with respect to the growth rate functions considered in the models in using qualitative hypotheses, cf. [5] . Furthermore, it was assumed that the species X_1 may be inhibited by the product S_2 that it produces itself while the species X_2 was simply limited by S_2. An example of such interactions was given by the anaerobic digestion in which mutualistic relationships allow certain classes of bacteria to coexist. A mutualistic relation has been also considered in [4]. See [6] for another model of coexistence in the chemostat.

Date: July 29, 2011.

2000 *Mathematics Subject Classification.* 92A15, 92A17, 34C15, 34C35, 34E18.

Key words and phrases. Syntrophic relationship; Mathematical modelling; Coexistence; Asymptotic stability; Anaerobic digestion.

In the actual paper, following [7], we revisit the model proposed in [5] in considering two main changes which significantly further extend the range of practical situations covered by the model. First, we assume that there is some S_2 in the influent. In other terms, the limiting substrate S_2 on which the species X_2 grows is not only produced by the species X_1 but is also available even if the species X_1 is not present. The second modification of the model is that the second species is supposed to be inhibited by an excess of S_1, the limiting substrate on which the first species grows. To illustrate the usefulness of such extensions of the original model by El Hajji et al. [5], the biological interpretation of these hypotheses within the context of the anaerobic process is given in the appendix.

The paper is organized as follows. In Section 2, we propose a modified system of four differential equations from the original model in [5]. The positive equilibria are determined and their local and global stability properties are established. In the case when the system has a unique positive equilibrium, the global asymptotic stability results are demonstrated through the Dulac's criterion that rules out the possibility of the existence of periodic solutions for the reduced planar system, the Poincaré-Bendixon Theorem and the Butler-McGehee Lemma. Hence, in this case, for every positive initial conditions, the solutions converge to the positive equilibrium point which corresponds to the coexistence of the two bacterial species as observed in real processes. Simulations are presented in Section 4, an example of a syntrophic relationship is given in Section 6 as a candidate for this model.

2. Mathematical model

Let S_1, X_1, S_2 and X_2 denote, respectively, the concentrations of the substrate, the first bacteria, the intermediate product, and the second bacteria present in the reactor at time t. We neglect all species-specific death rates and take into account the dilution rate only. Hence our model is described by the following system of ordinary differential equations :

$$
(1) \qquad \begin{cases} \dot{S}_1 &= D(S_1^{in} - S_1) - k_3\mu_1(S_1, S_2)X_1 \ , \\ \dot{X}_1 &= \mu_1(S_1, S_2)X_1 - DX_1 \ , \\ \dot{S}_2 &= D(S_2^{in} - S_2) - k_2\mu_2(S_1, S_2)X_2 + k_1\mu_1(S_1, S_2)X_1 \ , \\ \dot{X}_2 &= \mu_2(S_1, S_2)X_2 - DX_2 \ . \end{cases}
$$

Where $S_1^{in} > 0$ denotes the input concentration of substrate, $S_2^{in} > 0$ denotes the input concentration of the intermediate product and $D > 0$ is the dilution rate.

Assume that the functional response of each species $\mu_1, \mu_2 : \mathbb{R}_+^2 \to \mathbb{R}_+$ satisfies :

A1: $\mu_1, \mu_2 : \mathbb{R}_+^2 \to \mathbb{R}_+$, of class $\mathcal{C}^1$,
A2: $\mu_1(0, S_2) = 0, \qquad \mu_2(S_1, 0) = 0, \quad \forall\ (S_1, S_2) \in \mathbb{R}_+^2$,
A3: $\dfrac{\partial \mu_1}{\partial S_1}(S_1, S_2) > 0, \quad \dfrac{\partial \mu_1}{\partial S_2}(S_1, S_2) < 0, \quad \forall\ (S_1, S_2) \in \mathbb{R}_+^2$,
A4: $\dfrac{\partial \mu_2}{\partial S_1}(S_1, S_2) < 0, \quad \dfrac{\partial \mu_2}{\partial S_2}(S_1, S_2) > 0, \quad \forall\ (S_1, S_2) \in \mathbb{R}_+^2$.

Hypothesis **A2** expresses that no growth can take place for species X_1 without the substrate S_1 and that the intermediate product S_2 is obligate for the growth of species X_2. Hypothesis **A3** means that the growth of species X_1 increases with the substrate S_1 and it is inhibited by the intermediate product S_2 that it produces. Hypothesis **A4** means that the growth of species X_2 increases with intermediate product S_2 produced by species X_1 while it is inhibited by the

substrate S_1. Note that there is a syntrophic relationship between the two species.

We first scale system (1) using the following change of variables and notations :

$$s_1 = \frac{k_1}{k_3} S_1, \quad x_1 = k_1 X_1, \quad s_2 = S_2, \quad x_2 = k_2 X_2, \quad s_1^{in} = \frac{k_1}{k_3} S_1^{in}, \quad s_2^{in} = S_2^{in} \ .$$

The dimensionless equations thus obtained are :

$$\left\{ \begin{array}{rcl} \dot{s}_1 & = & D(s_1^{in} - s_1) - f_1(s_1, s_2) x_1 \ , \\ \dot{x}_1 & = & f_1(s_1, s_2) x_1 - D x_1 \ , \\ \dot{s}_2 & = & D(s_2^{in} - s_2) - f_2(s_1, s_2) x_2 + f_1(s_1, s_2) x_1 \ , \\ \dot{x}_2 & = & f_2(s_1, s_2) x_2 - D x_2 \ . \end{array} \right. \tag{2}$$

Where the functions $f_1, f_2 : \mathbb{R}_+^2 \to \mathbb{R}_+$ are defined by

$$f_1(s_1, s_2) = \mu_1(\frac{k_3}{k_1} s_1, s_2) \quad \text{and} \quad f_2(s_1, s_2) = \mu_2(\frac{k_3}{k_1} s_1, s_2).$$

Hypotheses **A1**–**A4** satisfied by the functions μ_1 and μ_2 translate in the following assumptions of the functions f_1 and f_2:

H1: $f_1, f_2 : \mathbb{R}_+^2 \to \mathbb{R}_+$, of class $\mathcal{C}^1$,
H2: $f_1(0, s_2) = 0, \qquad f_2(s_1, 0) = 0, \quad \forall\, (s_1, s_2) \in \mathbb{R}_+^2$,
H3: $\dfrac{\partial f_1}{\partial s_1}(s_1, s_2) > 0, \quad \dfrac{\partial f_1}{\partial s_2}(s_1, s_2) < 0, \quad \forall\, (s_1, s_2) \in \mathbb{R}_+^2$,
H4: $\dfrac{\partial f_2}{\partial s_1}(s_1, s_2) < 0, \quad \dfrac{\partial f_2}{\partial s_2}(s_1, s_2) > 0, \quad \forall\, (s_1, s_2) \in \mathbb{R}_+^2$.

$\mathbb{R}_+^4$, the closed non-negative cone in $\mathbb{R}^4$, is positively invariant under the solution map of system (2). More precisely

Proposition 1. *For every initial condition in $\mathbb{R}_+^4$, the solution of system (2) has positive components and is positively bounded and thus is defined for every positive t. The set*

$$\Omega = \left\{ (s_1, x_1, s_2, x_2) \in \mathbb{R}_+^4 : \ s_1 + x_1 = s_1^{in}, \quad s_2 + x_2 = x_1 + s_2^{in} \right\}$$

is a positive invariant attractor of all solutions of system (2).

Proof. The invariance of $\mathbb{R}_+^4$ is guaranteed by the fact that :

i. $s_1 = 0 \Rightarrow \dot{s}_1 = D\, s_1^{in} > 0$,
ii. $s_2 = 0 \Rightarrow \dot{s}_2 = D\, s_2^{in} + f_1(s_1, 0)\, x_1 > 0$,
iii. $x_i = 0 \Rightarrow \dot{x}_i = 0$ for $i = 1, 2$.

Next we have to prove that the solution is bounded. Let $z_1 = s_1 + x_1$, then $\dot{z}_1 = -D(z_1 - s_1^{in})$ from which one deduces :

$$s_1(t) + x_1(t) = s_1^{in} + (s_1(0) + x_1(0) - s_1^{in}) e^{-Dt} \ . \tag{3}$$

Thus $s_1(t)$ and $x_1(t)$ are positively bounded. Let $z_2 = s_2 + x_2 - x_1$, then $\dot{z}_2 = -D(z_2 - s_2^{in})$ from which one deduces:

$$s_2(t) + x_2(t) - x_1(t) = s_2^{in} + (s_2(0) + x_2(0) - x_1(0) - s_2^{in}) e^{-Dt} \ . \tag{4}$$

Thus $s_2(t)$ and $x_2(t)$ are positively bounded. Hence, the solution is defined for all positive t. From (3) and (4) we deduce that the set Ω is an invariant set which is an attractor. □

3. Restriction on the plane

The solutions of system (2) are exponentially convergent towards the set Ω and we are interested in the asymptotic behavior of these solutions. It is enough to restrict the study of the asymptotic behaviour of system (2) to Ω. In fact, thanks to Thieme's results [12], the asymptotic behaviour of the solutions of the restriction of (2) on Ω will be informative for the complete system, see Section 5. In this section we study the following reduced system which is simply the projection on the plane (x_1, x_2), of the restriction of system (2) on Ω.

$$\begin{cases} \dot{x}_1 = [\Phi_1(x_1, x_2) - D]\, x_1, \\ \dot{x}_2 = [\Phi_2(x_1, x_2) - D]\, x_2. \end{cases} \tag{5}$$

where

$$\Phi_1(x_1, x_2) = f_1\left(s_1^{in} - x_1, s_2^{in} + x_1 - x_2\right), \quad \Phi_2(x_1, x_2) = f_2\left(s_1^{in} - x_1, s_2^{in} + x_1 - x_2\right).$$

Thus, for (5) the state-vector (x_1, x_2) belongs to the following subset of the plane, see Fig. 1 :

$$\mathcal{S} = \left\{(x_1, x_2) \in {\mathbb{R}_+}^2 : 0 < x_1 \leq s_1^{in}, 0 < x_2 \leq x_1 + s_2^{in}\right\}.$$

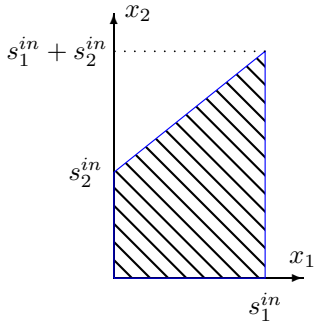

Figure 1. The set $\mathcal{S}$

The point $F^0 = (0, 0)$ is an equilibrium of (5). Besides this equilibrium point the system can have the following three types of equilibrium points.

- Boundary equilibria $F^1 = (\bar{x}_1, 0)$, where $x_1 = \bar{x}_1$ is a solution, if it exists, of equation

$$\Phi_1(x_1, 0) = D, \tag{6}$$

- Boundary equilibria $F^2 = (0, \tilde{x}_2)$, where $x_2 = \tilde{x}_2$ is a solution, if it exists, of equation

$$\Phi_2(0, x_2) = D, \tag{7}$$

- Positive equilibria $F^* = (x_1^*, x_2^*)$, where $x_1 = x_1^*$, $x_2 = x_2^*$ is a solution, if it exists, of the system of equations

$$\begin{cases} \Phi_1(x_1, x_2) = D \\ \Phi_2(x_1, x_2) = D. \end{cases} \tag{8}$$

We use the following notations

$$D_1 = f_1(s_1^{in}, s_2^{in}), \qquad D_2 = f_2(s_1^{in}, s_2^{in}).$$

The mapping $x_1 \mapsto \Phi_1(x_1, 0)$ is decreasing, and the mapping $x_1 \mapsto \Phi_2(x_1, 0)$ is increasing. If $D_1 > D_2$, there exists a unique real number ξ_1 satisfying $\Phi_1(\xi_1, 0) = \Phi_2(\xi_1, 0)$, since

$$\Phi_1(0, 0) = D_1 > D_2 = \Phi_2(0, 0), \text{ and } \Phi_1(s_1^{in}, 0) = 0 < \Phi_2(s_1^{in}, 0).$$

We denote by $D_3 \in]D_2, D_1[$ the unique real number (see Figure 2, right) such that:

$$\Phi_1(\xi_1, 0) = \Phi_2(\xi_1, 0) = D_3.$$

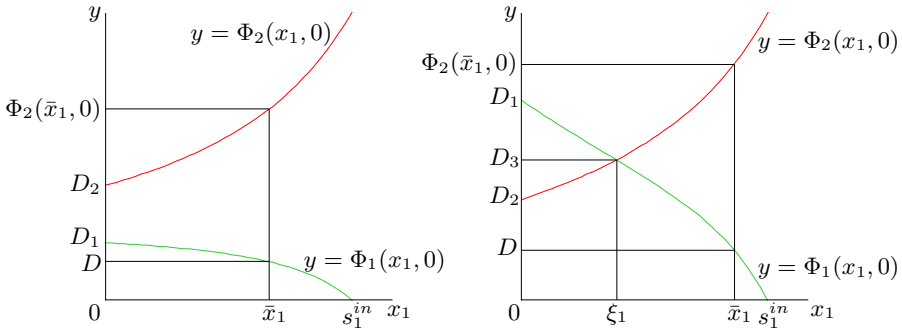

FIGURE 2. Existence and uniqueness of $\bar{x}_1$. On the left, the case $D_1 < D_2$: $\Phi_2(\bar{x}_1, 0) > D$ for all $D < D_1$. On the right, the case $D_1 > D_2$: $\Phi_2(\bar{x}_1, 0) > D$ if and only if $D < D_3$.

The mapping $x_2 \mapsto \Phi_1(0, x_2)$ is increasing, and the mapping $x_2 \mapsto \Phi_2(0, x_2)$ is decreasing. Hence, if $D_1 < D_2$, there exists a unique real number ξ_2 satisfying $\Phi_1(0, \xi_2) = \Phi_2(0, \xi_2)$, since

$$\Phi_2(0, 0) = D_2 > D_1 = \Phi_1(0, 0), \text{ and } \Phi_2(0, s_2^{in}) = 0 < \Phi_1(0, s_2^{in}).$$

We denote by $D_4 \in]D_1, D_2[$ the unique real number (see Figure 3, right) such that:

$$\Phi_1(0, \xi_2) = \Phi_2(0, \xi_2) = D_4.$$

The nature of the trivial equilibrium point F^0 is given in the following lemma.

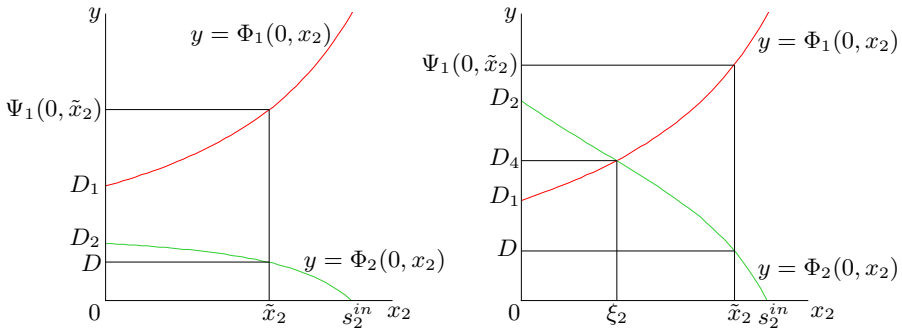

FIGURE 3. Existence and uniqueness of $\tilde{x}_2$. On the left, the case $D_2 < D_1$: $\Phi_1(0, \tilde{x}_2) > D$ for all $D < D_2$. On the right, the case $D_2 > D_1$: $\Phi_1(0, \tilde{x}_2) > D$ if and only if $D < D_4$.

Lemma 1. *If $D > \max(D_1, D_2)$ then F^0 is a stable node. If $\min(D_1, D_2) < D < \max(D_1, D_2)$ then F^0 is a saddle point. If $D < \min(D_1, D_2)$ then F^0 is an unstable node.*

Proof. The Jacobian matrix J of (5), at point (x_1, x_2), is given by:

$$J = \begin{bmatrix} -\dfrac{\partial f_1}{\partial s_1}x_1 + \dfrac{\partial f_1}{\partial s_2}x_1 + f_1 - D & -\dfrac{\partial f_1}{\partial s_2}x_1 \\ -\dfrac{\partial f_2}{\partial s_1}x_2 + \dfrac{\partial f_2}{\partial s_2}x_2 & -\dfrac{\partial f_2}{\partial s_2}x_2 + f_2 - D \end{bmatrix}.$$

where the functions are evaluated at $\left(s_1^{in} - x_1, s_2^{in} + x_1 - x_2\right)$. The Jacobian matrix at F^0 is given by:

$$J^0 = \begin{bmatrix} f_1(s_1^{in}, s_2^{in}) - D & 0 \\ 0 & f_2(s_1^{in}, s_2^{in}) - D \end{bmatrix}$$

The eigenvalues are $D_1 - D$ and $D_2 - D$. Thus, if $D > \max(D_1, D_2)$ then F^0 is a stable node. It is an unstable node if $D < \min(D_1, D_2)$. It is a saddle point if $\min(D_1, D_2) < D < \max(D_1, D_2)$. □

The conditions of existence of the boundary equilibria F^1 and F^2, and their nature, are stated in the following lemmas.

Lemma 2. *An equilibrium $F^1 = (\bar{x}_1, 0)$ exists if and only if $D < D_1$. If it exists then it the unique equilibrium on the positive x_1 semi-axis. If $D_1 < D_2$ then F^1 is a saddle point for all $D < D_1$. If $D_2 < D_1$, then F^1 is a saddle point for all $0 < D < D_3$ and a stable node for all $D_3 < D < D_1$.*

Proof. An equilibrium $F^1 = (\bar{x}_1, 0)$ exists if and only if $x_1 = \bar{x}_1 \in]0, s_1^{in}[$ is a solution of (6). Let $\psi_1(x_1) = \Phi_1(x_1, 0)$. Then

$$\psi_1'(x_1) = -\frac{\partial f_1}{\partial s_1}(s_1^{in} - x_1, s_2^{in} + x_1) + \frac{\partial f_1}{\partial s_2}(s_1^{in} - x_1, s_2^{in} + x_1).$$

By assumption **H3**, $\psi_1'(x_1) < 0$. Since $\psi_1(0) = D_1$, and $\psi_1(s_1^{in}) = 0$, equation (6) admits a solution in the interval $]0, s_1^{in}[$ if and only if $D < D_1$. If this condition is satisfied then (6) admits a unique solution since the function $\psi_1(.)$ is decreasing, see Figure 2. The Jacobian matrix at F^1 is given by:

$$J^1 = \begin{bmatrix} -\dfrac{\partial f_1}{\partial s_1}\bar{x}_1 + \dfrac{\partial f_1}{\partial s_2}\bar{x}_1 & -\dfrac{\partial f_1}{\partial s_2}\bar{x}_1 \\ 0 & f_2 - D \end{bmatrix}$$

where the functions are evaluated at $(s_1^{in} - \bar{x}_1, s_2^{in} + \bar{x}_1)$. The eigenvalues are

$$f_2(s_1^{in} - \bar{x}_1, s_2^{in} + \bar{x}_1) - D = \Phi_2(\bar{x}_1, 0) - D, \text{ and } -\frac{\partial f_1}{\partial s_1}\bar{x}_1 + \frac{\partial f_1}{\partial s_2}\bar{x}_1 < 0.$$

Thus F^1 is a saddle point if $\Phi_2(\bar{x}_1, 0) > D$. If $D_1 < D_2$, this condition is satisfied for all $D < D_1$. If $D_2 < D_1$, it is statisfied for all $0 < D < D_3$, see Figure 2. F^1 is a stable node if $D_3 < D < D_1$ and $D_2 < D_1$. □

Lemma 3. *An equilibrium $F^2 = (0, \tilde{x}_2)$ exists if and only if $D < D_2$. If it exists then it the unique equilibrium on the positive x_2 semi-axis. If $D_2 < D_1$ then F^2 is a saddle point for all $D < D_2$. If $D_1 < D_2$, then F^2 is a saddle point for all $0 < D < D_4$ and a stable node for all $D_4 < D < D_2$.*

Proof. An equilibrium $F^2 = (0, \tilde{x}_2)$ exists if and only if $x_2 = \tilde{x}_2 \in]0, s_2^{in}[$ is a solution of (7). Let $\psi_2(x_2) = \Phi_2(0, x_2)$. Then

$$\psi_2'(x_2) = -\frac{\partial f_1}{\partial s_2}(s_1^{in}, s_2^{in} - \tilde{x}_2).$$

By assumption **H4**, $\psi_2'(x_2) < 0$. Since $\psi_2(0) = D_2$, and $\psi_2(s_2^{in}) = 0$, equation (7) admits a solution in the interval $]0, s_2^{in}[$ if and only if $D < D_2$. If this condition is satisfied then (7) admits a unique solution since the function $\psi_2(.)$ is decreasing, see Figure 3. The Jacobian matrix at F^2 is given by:

$$J^2 = \begin{bmatrix} f_1 - D & 0 \\ -\dfrac{\partial f_2}{\partial s_1}\tilde{x}_2 + \dfrac{\partial f_2}{\partial s_2}\tilde{x}_2 & -\dfrac{\partial f_2}{\partial s_2}\tilde{x}_2 \end{bmatrix}$$

where the functions are evaluated at $(s_1^{in}, s_2^{in} - \tilde{x}_2)$. The eigenvalues are

$$f_1(s_1^{in}, s_2^{in} - \tilde{x}_2) - D = \Phi_1(0, \tilde{x}_2) - D, \text{ and } -\frac{\partial f_2}{\partial s_2}\tilde{x}_2 < 0.$$

Thus F^2 is a saddle point if $\Phi_1(0, \tilde{x}_2) > D$. If $D_2 < D_1$, this condition is satisfied for all $D < D_2$. If $D_1 < D_2$, it is statisfied for all $0 < D < D_4$, see Figure 3. F^2 is a stable node if $D_4 < D < D_2$ and $D_1 < D_2$. □

Let us discuss now the conditions of existence of positive equilibria F^*, and their number. An equilibrium $F^* = (x_1^*, x_2^*)$ exists if and only if $x_1 = x_1^*$, $x_2 = x_2^*$ is a solution of (8) lying in $\mathcal{S}$. One has

$$\frac{\partial \Phi_1}{\partial x_2} = -\frac{\partial f_1}{\partial s_2}(s_1^{in} - x_1, s_2^{in} + x_1 - x_2).$$

By assumption **H3**, this partial derivative is positive. Hence, equation $\Phi_1(x_1, x_2) = D$ defines a function $x_2 = F_1(x_1)$ such that $F_1(\bar{x}_1) = 0$ when $D < D_1$. Recall that $x_1 = \bar{x}_1$ is the solution of (6) which, according to Lemma 2 exists and is unique, if and only if $D < D_1$. One has

$$F_1'(x_1) = -\frac{\frac{\partial \Phi_1}{\partial x_1}(x_1, F_1(x_1))}{\frac{\partial \Phi_1}{\partial x_2}(x_1, F_1(x_1))} = \frac{-\frac{\partial f_1}{\partial s_1} + \frac{\partial f_1}{\partial s_2}}{\frac{\partial f_1}{\partial s_2}} = 1 - \frac{\frac{\partial f_1}{\partial s_1}}{\frac{\partial f_1}{\partial s_2}} > 1.$$

Hence the function F_1 is increasing. Since $\Phi_1(s_1^{in}, 0) = 0$, the graph Γ_1 of F_1 has no intersection with the right boundary of the domain $\mathcal{S}$, defined by $x_1 = s_1^{in}$. This graph separates $\mathcal{S}$ in two regions denoted as the left and right sides of Γ_1, see Figure 4. One has also

$$\frac{\partial \Phi_2}{\partial x_2} = -\frac{\partial f_2}{\partial s_2}(s_1^{in} - x_1, s_2^{in} + x_1 - x_2).$$

By assumption **H3**, this partial derivative is positive. Hence, equation $\Phi_2(x_1, x_2) = D$ defines a function $x_2 = F_2(x_1)$ such that $F_2(0) = \tilde{x}_2$ when $D < D_2$. Recall that $x_2 = \tilde{x}_2$ is the solution of (7) which, according to Lemma 3 exists and is unique, if and only if $D < D_2$. One has

$$F_2'(x_1) = -\frac{\frac{\partial \Phi_2}{\partial x_1}(x_1, F_2(x_1))}{\frac{\partial \Phi_2}{\partial x_2}(x_1, F_2(x_1))} = \frac{-\frac{\partial f_2}{\partial s_1} + \frac{\partial f_2}{\partial s_2}}{\frac{\partial f_2}{\partial s_2}} = 1 - \frac{\frac{\partial f_2}{\partial s_1}}{\frac{\partial f_2}{\partial s_2}} > 1.$$

Hence the function F_2 is increasing. Since $\Phi_2(x_1, s_2^{in} + x_1) = 0$, the graph Γ_2 of F_2 has no intersection with the top boundary of the domain $\mathcal{S}$, defined by $x_2 = s_2^{in} + x_1$. Thus the point at the very right of Γ_2 lies necessarily on the right boundary of $\mathcal{S}$, defined by $x_1 = s_1^{in}$. Hence it lies on the right side of Γ_1, see Figure 4.

The graphs Γ_1 and Γ_2 can intersect or not, see Figures 4, 5 and 6. If they intersect at some point $F^* = (x_1^*, x_2^*)$ then F^* is a positive equilibbrium. If the point A at the very left of Γ_2

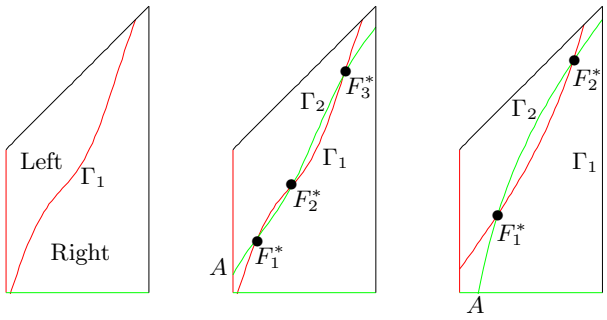

FIGURE 4. On the left, the left and right sides of Γ_1. On the center, the point A at the very left of Γ_2 lies on left side of Γ_1: there are generically an odd number of intersections (3 in this example). On the right, the point A at the very left of Γ_2 lies on right side of Γ_1: there are generically an even number of intersections (2 in this example).

lies on left side of Γ_1 then Γ_1 and Γ_2 intersect in at least one point $F^* = (x_1^*, x_2^*)$. They can have multiple intersections. Generically they have an odd number of intersections (see Figure 4, center). If the point A at the very left of Γ_2 lies on right side of Γ_1 then Γ_1 and Γ_2 can intersect or not. Generically they have an even number of intersections (see Figure 4, right). The nature of a positive equilibrium F^* is stated in the following lemmas.

Lemma 4. *If an equilibrium $F^* = (x_1^*, x_2^*)$ exists then it is a stable node if $F_1'(x_1^*) > F_2'(x_1^*)$. It is a saddle point if the opposite inequality is satisfied.*

Proof. The Jacobian matrix at F^* is given by:

$$J^* = \begin{bmatrix} -\dfrac{\partial f_1}{\partial s_1}x_1^* + \dfrac{\partial f_1}{\partial s_2}x_1^* & -\dfrac{\partial f_1}{\partial s_2}x_1^* \\ -\dfrac{\partial f_2}{\partial s_1}x_2^* + \dfrac{\partial f_2}{\partial s_2}x_2^* & -\dfrac{\partial f_2}{\partial s_2}x_2^* \end{bmatrix}$$

where the derivatives are evaluated at $(s_1^{in} - x_1^*, s_2^{in} + x_1^* - x_2^*)$. Notice that

$$\mathrm{tr}(J^*) = -\frac{\partial f_1}{\partial s_1}x_1^* + \frac{\partial f_1}{\partial s_2}x_1^* - \frac{\partial f_2}{\partial s_2}x_2^* < 0$$

and

$$\det(J^*) = x_1^*x_2^*\left[\frac{\partial f_1}{\partial s_1}\frac{\partial f_2}{\partial s_2} - \frac{\partial f_1}{\partial s_2}\frac{\partial f_2}{\partial s_1}\right] = x_1^*x_2^*\frac{\partial f_1}{\partial s_2}\frac{\partial f_2}{\partial s_2}\left[F_2'(x_1^*) - F_1'(x_1^*)\right].$$

By Assumptions **H3** and **H4**, the product of the partial derivatives is negative. Therefore, the determinant is positive if $F_1'(x_1^*) > F_2'(x_1^*)$ and negative if the opposite inequality is satisfied. Hence the equilibrium $F^* = (x_1^*, x_2^*)$ is a stable node if $F_1'(x_1^*) > F_2'(x_1^*)$. It is a saddle point if the opposite inequality is satisfied. □

The number of equilibria of (5) and their nature are summarized in the next theorem.

Theorem 1. (1) *If $D < \min(D_1, D_2)$ then (5) admits the trivial equilibrium F^0 which is an unstable node, the boundary equilibria F^1 and F^2 which are saddle points, and at least one positive equilibrium F^*. If F^* is the unique positive equilibrium then it is a stable node. Generically, the system has an odd number of positive equilibria which are*

alternatively stable nodes and saddle points, the one at the very left of these positive equilibria is a stable node.

(2) *If* $\min(D_1, D_2) < D < \max(D_1, D_2)$*, four subcases must be distinguished*
   (a) *If* $D_1 < D_2$ *and* $D_1 < D < D_4$ *then (5) admits the trivial and boundary equilibria* F^0 *and* F^2*, which are saddle points and at least one positive equilibrium* F^**. If* F^* *is the unique positive equilibrium then it is a stable node. Generically, the system has an odd number of positive equilibria which are alternatively stable nodes and saddle points, the one at the very left of these positive equilibria is a stable node.*
   (b) *If* $D_1 < D_2$ *and* $D_4 < D < D_2$ *then (5) admits the trivial equilibrium* F^0*, which is a saddle point, and the boundary equilibrium* F^2*, which is a stable node. Generically, the system can have an even number of positive equilibria which are alternatively saddle points and stable nodes, the one at the very left of these positive equilibria is a saddle point.*
   (c) *If* $D_2 < D_1$ *and* $D_2 < D < D_3$ *then (5) admits the trivial and boundary equilibria* F^0 *and* F^1 *which are saddle points and at least one positive equilibrium* F^**. If* F^* *is the unique positive equilibrium then it is a stable node. Generically, the system has an odd number of positive equilibria which are alternatively stable nodes and saddle points, the one at the very left of these positive equilibria is a stable node.*
   (d) *If* $D_2 < D_1$ *and* $D_3 < D < D_1$ *then (5) admits the trivial equilibrium* F^0*, which is a saddle point, and the boundary equilibrium* F^1*, which is a stable node. Generically, the system can have an even number of positive equilibria which are alternatively saddle points and stable nodes, the one at the very left of these positive equilibria is a saddle point.*

(3) *If* $D > \max(D_1, D_2)$ *then (5) admits the trivial equilibrium* F^0 *which is a stable node. Generically, the system can have an even number of positive equilibria which are alternatively saddle points and stable nodes, the one at the very left of these positive equilibria is a saddle point.*

4. Growth functions of Monod type

In this section we consider growth functions f_1 and f_2 of the following form

$$f_1(s_1, s_2) = \frac{m_1 s_1}{(K_1 + s_1)(L_1 + s_2)}, \qquad f_2(s_1, s_2) = \frac{m_2 s_1}{(K_2 + s_1)(L_2 + s_2)}. \tag{9}$$

Such functions are simply the product of a Monod function in s_1 by a decreasing functions of s_2. Such functions are currently used in biotechnology when the growth of a functional species is limited by a substrate while inhibited by another one. Such situations are common in water treatment technology like in the denitrification (limited by the nitrate and inhibited by the dissolved oxygen) or in the anoxic or anaerobic hydrolysis (limited by the slowly biodegradable substrates while inhibited by an excess of oxygen) processes which are modeled this way (cf. [13]).

One can readily check that (9) satisfy Assumptions **H1** to **H4**. By straighforward calculations one has

$$F_1(x_1) = \frac{-Dx_1^2 + \left[m_1 + D\left(K_1 - L_1 + s_1^{in} - s_2^{in}\right)\right] - m_1 s_1^{in} + D\left(K_1 + s_1^{in}\right)\left(L_1 + s_2^{in}\right)}{D(K_1 + s_1^{in} - x_1)}$$

$$F_2(x_1) = \frac{Dx_1^2 + \left[m_2 + D\left(L_2 - K_2 + s_2^{in} - s_1^{in}\right)\right] + m_2 s_2^{in} - D\left(K_2 + s_1^{in}\right)\left(L_2 + s_2^{in}\right)}{m_2 - D(K_2 + s_1^{in} - x_1)}$$

Hence equation $F_1(x_1) = F_2(x_1)$ giving the abscissa of positive equilibria is an algebraic equation of degree 2. Thus, it cannot have more than two solutions. Hence, the situation depicted on the

center of Figure 4, of three positive equilibria, is excluded. However, the situation depicted in the right of Figure 4, with two positive equilibria can occur. For instance, consider the following

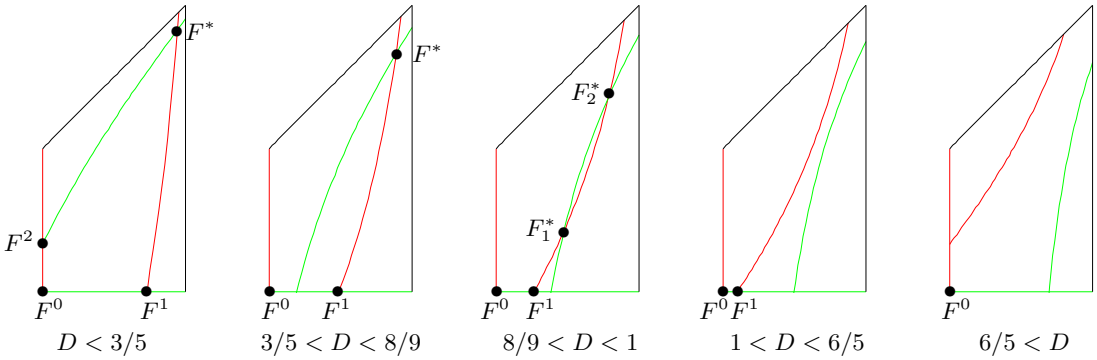

FIGURE 5. Relative positions of the isocline $\dot{x}_1 = 0$ (in red) and $\dot{x}_2 = 0$ (in green).

values of the parameters

$$m_1 = 8, \quad m_2 = 4, \quad K_1 = L_2 = 1, \quad L_1 = K_2 = 2, \quad s_1^{in} = s_2^{in} = 3 \tag{10}$$

Then

$$D_1 = 6/5, \qquad D_3 = 8/9, \qquad D_2 = 3/5.$$

There is another bifurcation value, $D = 1$ which correspond to the case when the graphs Γ_1 and Γ_2 are tangent, see Figure 6. For this example five cases can occur, see Figure 5:

Proposition 2. *Consider system (5) where f_1 and f_2 are given by (9) with parameters (10). Then*

1. *when $D < 3/5$, the system has four equilibria, F^0 which is an unstable node, F^1 and F^2, which are saddle points and F^*, which is a stable node. This is case (1) of Theorem 1, with a unique positive equilibrium.*
2. *when $3/5 < D < 8/9$, the system has three equilibria, F^0 and F^1, which are saddle points and F^*, which is a stable node. This is case (2.c) of Theorem 1, with a unique positive equilibrium.*
3. *when $8/9 < D < 1$, the system has four equilibria, F^0 and F_1^*, which are saddle points and F^1 and F_2^*, which are stable nodes. This is case (2.d) of Theorem 1, with two positive equilibria.*
4. *when $1 < D < 6/5$, the system has two equilibria, F^0, which is a saddle point and F^1 which is a stable node. This is case (2.d) of Theorem 1, with no positive equilibrium.*
5. *when $D > 6/5$, the system has one equilibrium, F^0, which is a stable node. This is case (3) of Theorem 1, with no positive equilibrium.*

In the case when $8/9 < D < 1$ a bistability phenomenon occurs. According to the initial condition, both species can coexist at equilibrium F_2^*, or species x_2 goes to extinction at equilibrium F^1. This phenomenon is illustarted numerically with $D = 0.95$ in Figure 7.

For the following values of the parameters

$$m_1 = 8, \quad m_2 = 7, \quad K_1 = K_2 = L_2 = 1, \quad L_1 = 3/2, \quad s_1^{in} = s_2^{in} = 3 \tag{11}$$

the bifurcational values are $D_1 = 4/3$ and $D_2 = 21/16$. If $D > \max(D_1, D_2)$, for instance for $D = 3/2$, one obtains a bistability phenomenon corresponding to case (3) of Theorem 1, with two positive equilibria. According to the initial condition, both species can coexist at equilibrium F_2^*,

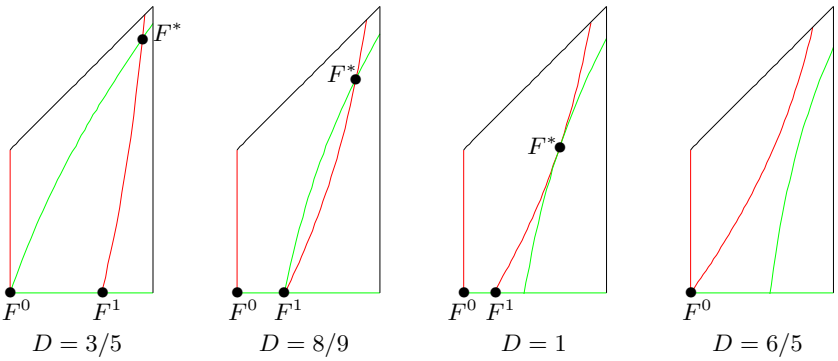

FIGURE 6. The non hyperbolic cases. When $D = 6/5$, F^0 and F^2 coalesce. When $D = 8/9$, F_1^* and F^1 coalesce (saddle node bifurcation). When $D = 1$, F_1^* and F_2^* coalesce (saddle node bifurcation). When $D = 6/5$, F^0 and F^2 coalesce.

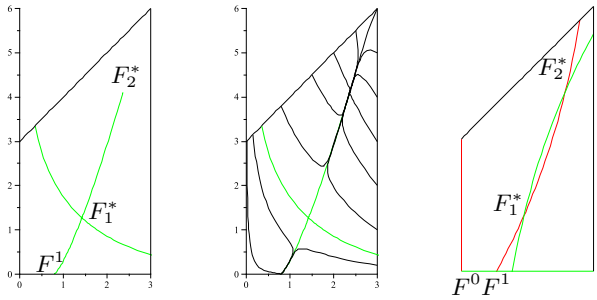

FIGURE 7. Numerical solutions in the bistability case $D = 0.95$ and parameters values (10). On the left, the separatrix (in green) of the saddle point F_1^* separate the domain $\mathcal{S}$ in two region which are the basins of attraction of the boundary equilibrium point F^1 and the positive equilibrium point F_2^*. On the center, the phase portrait. On the right, the isoclines.

or both species go to extinction at equilibrium F^0. This phenomenon is illustarted numerically in Figure 8.

5. Global analysis

Let us establish first that (5) admits no periodic orbit nor polycycle inside $\mathcal{S}$

Theorem 2. *There are no periodic orbits nor polycycles inside $\mathcal{S}$.*

Proof. . Consider a trajectory of (5) belonging to $\mathcal{S}$. Let us transform the system (5) through the change of variables $\xi_1 = \ln(x_1)$, $\xi_2 = \ln(x_2)$. Then one obtains the following system :

$$\left\{\begin{array}{rcl} \dot{\xi_1} & = & h_1(\xi_1,\xi_2) := f_1(s_1^{in} - e^{\xi_1}, s_2^{in} + e^{\xi_1} - e^{\xi_2}) - D, \\ \dot{\xi_2} & = & h_1(\xi_1,\xi_2) := f_2(s_1^{in} - e^{\xi_1}, s_2^{in} + e^{\xi_1} - e^{\xi_2}) - D. \end{array}\right. \tag{12}$$

We have

$$\frac{\partial h_1}{\partial \xi_1} + \frac{\partial h_2}{\partial \xi_2} = -e^{\xi_1}\frac{\partial f_1}{\partial s_1} + e^{\xi_1}\frac{\partial f_1}{\partial s_2} - e^{\xi_2}\frac{\partial f_2}{\partial s_2} < 0.$$

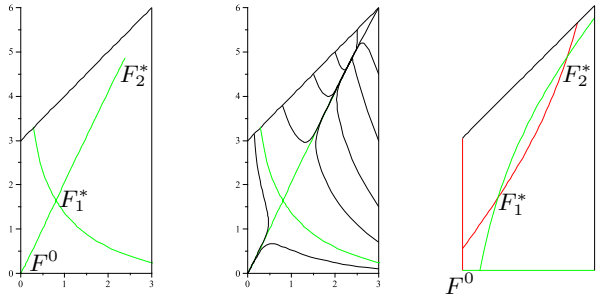

FIGURE 8. Numerical solutions in the bistability case $D = 1.5$ and parameters values (11). On the left, the separatrix (in green) of the saddle point F_1^* separate the domain $\mathcal{S}$ in two region which are the basins of attraction of the boundary equilibrium point F^0 and the positive equilibrium point F_2^*. On the center, the phase portrait. On the right, the isoclines.

From Dulac criterion [11], we deduce that the system (12) has no periodic trajectory. Hence (5) has no periodic orbit in $\mathcal{S}$. □

Theorem 3. *Assume that system (5) has at most one positive quilibrium F^*, then for every initial condition in $\mathcal{S}$, the trajectories of system (5) converge asymptotically to :*

- F^* *if* $D < \min(D_1, D_2)$.
- F^* *if* $D_1 < D_2$ *and* $D_1 < D < D_4$
- F^2 *if* $D_1 < D_2$ *and* $D_4 < D < D_2$.
- F^* *if* $D_2 < D_1$ *and* $D_2 < D < D_3$.
- F^1 *if* $D_2 < D_1$ *and* $D_3 < D < D_1$.
- F^0 *if* $\max(D_1, D_4) < D$.

Proof. We restrict the proof to the situation where $D < \min(D_1, D_2)$. The other cases can be done similarly. Let $x_1(0) > 0, x_2(0) > 0$ and ω the ω-limit set of $(x_1(0), x_2(0))$. ω is an invariant compact set and $\omega \subset \bar{\mathcal{S}}$. Assume that ω contains a point M on the x_1x_2 axis :

- M can't be F^0 because F^0 is an unstable node and can't be a part of the ω-limit set of $(x_1(0), x_2(0))$,
- If $M \in]\bar{x}_1, s_1^{in}] \times \{0\}$ (respectively $M \in \{0\}\times]\bar{x}_2, s_2^{in}]$). As ω is invariant then $\gamma(M) \subset \omega$ which is impossible because ω is bounded and $\gamma(M) =]\bar{x}_1, +\infty[\times\{0\}$ (respectively $\gamma(M) = \{0\}\times]\bar{x}_2, +\infty[$),
- If $M \in]0, \bar{x}_1[\times\{0\}$ (respectively $M \in \{0\}$, $\times]0, \bar{x}_2[$). ω contains $\gamma(M) =]0, \bar{x}_1[\times\{0\}$ (respectively $\gamma(M) = \{0\}\times]0, \bar{x}_2[$). As ω is a compact, then it contains the adherence of $\gamma(M)$, $[0, \bar{x}_1] \times \{0\}$ (respectively $\{0\} \times [0, \bar{x}_2]$). In particular, ω contains F^0 which is impossible,
- If $M = F^1$ (respectively $M = F^2$). ω is not reduced to F^1 (respectively to F^2). By Butler-McGehee theorem, ω contains a point P of $(0, +\infty) \times \{0\}$ other that F^1 (respectively of $\{0\} \times (0, +\infty)$ other that F^2) which is impossible.

Finally, the ω-limit set don't contain any point on the x_1x_2 axis. System (5) has no periodic orbit inside $\mathcal{S}$. Using the Poincar-Bendixon Theorem [11], F^* is a globally asymptotically stable equilibrium point for system (5). □

Theorem 4. *Assume that system (5) has at most one positive quilibrium F^*, then for every initial condition in $\mathbb{R}^4_+$, the trajectories of system (2) converge asymptotically to:*

- E^* *if* $D < \min(D_1, D_4)$.
- E^* *if* $D_1 < D_2$ *and* $D_1 < D < D_4$.
- E^2 *if* $D_1 < D_2$ *and* $D_4 < D < D_2$.
- E^* *if* $D_2 < D_1$ *and* $D_2 < D < D_3$.
- E^1 *if* $D_2 < D_1$ *and* $D_3 < D < D_1$.
- E^0 *if* $\max(D_1, D_2) < D$.

Proof. Let $(s_1(t), x_1(t), s_2(t), x_2(t))$ be a solution of (2). From (3) and (4) we deduce that

$$s_1(t) = s_1^{in} - x_1(t) + K_1 e^{-Dt} \quad \text{and} \quad s_2(t) = s_2^{in} + x_1(t) - x_2(t) + K_2 e^{-Dt},$$

where $K_1 = s_1(0) + x_1(0) - s_1^{in}$ and $K_2 = s_2(0) + x_2(0) - x_1(0) - s_2^{in}$. Hence $(x_1(t), x_2(t))$ is a solution of the nonautonomous system of two differential equations :

$$\left\{ \begin{array}{rcl} \dot{x}_1 & = & \left[f_1\left(s_1^{in} - x_1 + K_1 e^{-Dt}, s_2^{in} + x_1 - x_2 + K_2 e^{-Dt}\right) - D \right] x_1, \\ \\ \dot{x}_2 & = & \left[f_2\left(s_1^{in} - x_1 + K_1 e^{-Dt}, s_2^{in} + x_1 - x_2 + K_2 e^{-Dt}\right) - D \right] x_2. \end{array} \right. \tag{13}$$

This is an asymptotically autonomous differential system which converge to the autonomous system (5). The set Ω is attractor of all trajectories in $\mathbb{R}^4_+$ and the phase portrait of system reduced to Ω (5) contains only locally stable nodes, unstable nodes, saddle points and no trajectory joining two saddle points. Thus we can apply Thiemes's results [12] and conclude that the asymptotic behaviour of the solution of the complete system (13) is the same that the asymptotic behaviour described for the reduced system (5) and the main result is then deduced. □

6. The anaerobic digestion process : An example of a synthrophic relationship

"Methane fermentation" or "anaerobic digestion" is a process that converts organic matter into a gaseous mixture mainly composed of methane and carbon dioxide (CH_4 and CO_2) through the action of a complex bacterial ecosystem (cf. Fig.9). It is often used for the treatment of concentrated wastewaters or to stabilize the excess sludge produced in wastewater treatment plants into more stable products. There is also considerable interest in plant-biomass-fed digesters, since the produced methane can be valorized as a source of energy. It is usually considered that a number of metabolic groups of bacteria are involved sequencially.

One specific characteristic of the anaerobic process is that within such groups, there exists populations exhibiting obligatory mutualistic relationships. Such a syntrophic relationship is necessary for the biological reactions to be thermodynamically possible. In the first steps of the reactions (called "acidogenesis"), some hydrogen is produced. In El Hajji et al.[5], this production of hydrogen at this reaction step was neglected (compare Fig.9 with Fig.1 of [5]). This hypothesis constitue the first novelty with respect to [5]. It is to be noticed that an excess of hydrogen in the medium inhibits the growth of another bacterial group called "acetogenic bacteria". Their association with H_2 consuming bacteria is thus necessary for the second step of the reaction to be fulfilled. Such a syntrophic relationship has been pointed out in a number of experimental works (cf. for instance the seminal work by [3]). Let us consider the subsystem of the anaerobic system where the VFA (for Volatile Fatty Acids) are transformed into H_2, CH_4 and $CO2$. We can formalize the corresponding biological reactions as a first bacterial consortium X_1 (the acetogens) transforming S_1 (the VFA) into S_2 (the hydrogen) and acetate (cf. Fig.9). Then, a second species X_2 (the hydrogenotrophic-methanogenic bacteria) grows on S_2. In practice, acetogens are inhibited by an excess of hydrogen and methanogens by an excess of VFA. Thus, it is further assumed that X_1 is inhibitied by S_2 and X_2 by S_1. The last inhibition relationship constiute the second novelty with respect to [5]. This situation is precisely the one considered within the model (1).

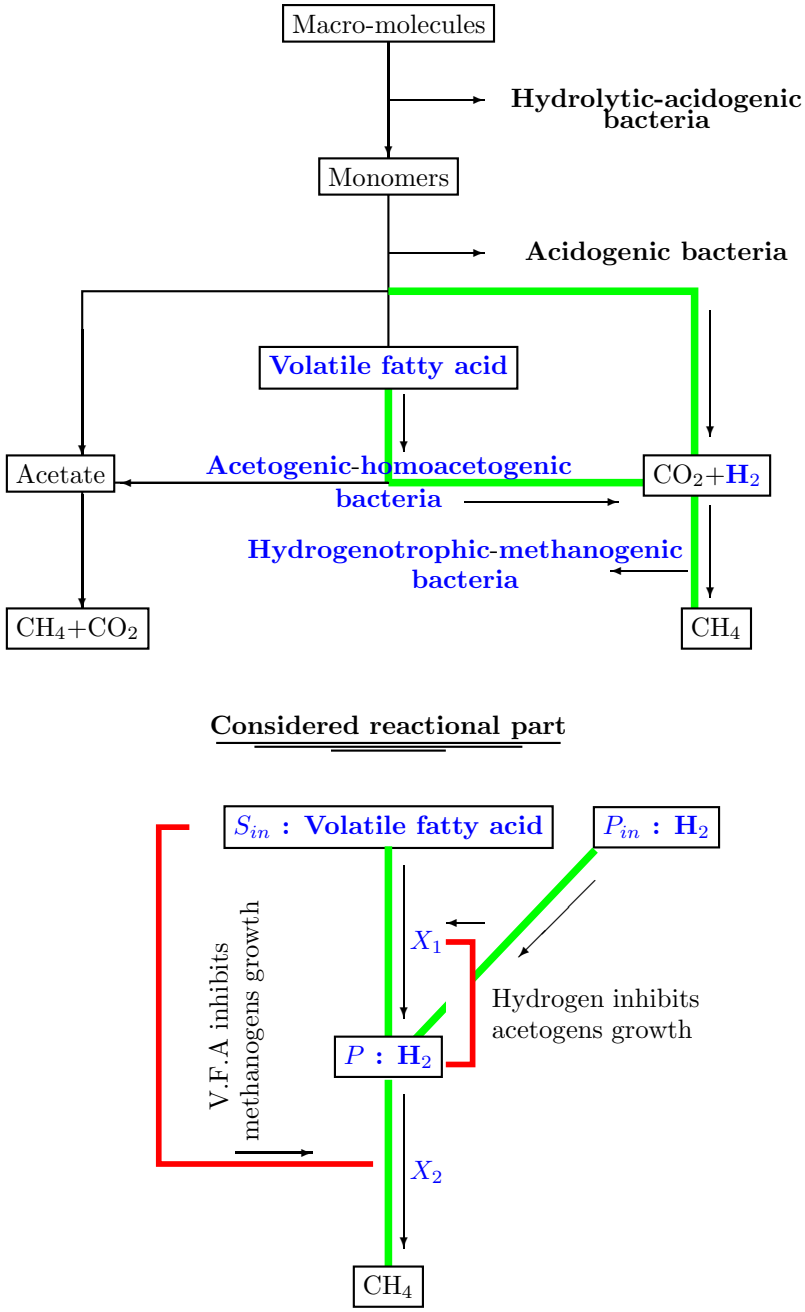

FIGURE 9. Anaerobic fermentation process

We have proposed a mathematical model involving a syntrophic relationship of two bacteria. It results from this analysis that, under general and natural assumptions of monotonicity on the functional responses, the stable asymptotic coexistence of the two bacteria is possible.

Acknowledgements

The authors acknowledge Inra and Inria for financial support.

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Tewfik Sari, Universite de Haute Alsace, LMIA, 4 rue des Frères Lumière, 68093 Mulhouse, & Inra-Inria Modemic research team, UMR Mistea, SupaAgro, 2 place Viala, 34060 Montpellier, France.
E-mail address: Tewfik.Sari@uha.fr

Miled EL Hajji, ISSAT, Universit de Kairouan 3000 Avenue Beit el hikma, Kairouan Tunisie
E-mail address: miled.elhajji@enit.rnu.tn

Jérôme Harmand, LBE-INRA, UR050, Avenue des Étangs, 11100 Narbonne, France & Inra-Inria Modemic research team, UMR Mistea, SupaAgro, 2 place Viala, 34060 Montpellier, France.
E-mail address: harmand@supagro.inra.fr